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Effects of Combinations of β-Lactams, Daptomycin, Gentamicin, and Glycopeptides against Glycopeptide-Resistant Enterococci

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Activities of combinations of beta-lactams, daptomycin, gentamicin, teicoplanin, and vancomycin against 11 clinical isolates of *Enterococcus faecium* highly resistant to glycopeptides, three plasmid-cured derivatives, eight *E. faecalis* and *E. faecium* transconjugants, and two susceptible recipient strains were tested. A marked synergy between penicillins or imipenem and glycopeptides against the glycopeptide-resistant strains but not against the glycopeptide-susceptible strains was observed by the double-disk agar diffusion assay. The synergy of combinations of amoxicillin, imipenem, penicillin G, or piperacillin with vancomycin or teicoplanin against resistant strains was confirmed by the checkerboard technique. The fractional inhibitory concentration indexes were generally below 0.25, except for one strain of *E. faecium* resistant to high levels of penicillin G. However, the combinations were not bactericidal as tested by time-killing experiments, and high concentrations (64 μg/ml) of amoxicillin, penicillin G, or piperacillin combined with 8 μg of vancomycin or teicoplanin per ml tended to be antagonistic. Addition of 4 μg of gentamicin per ml to these combinations enhanced their bactericidal effect, but they occasionally remained slightly less effective than beta-lactams associated with gentamicin. The combination of 10 μg of daptomycin per ml with gentamicin was bactericidal after 6 h against 11 glycopeptide-resistant strains.

Severe infections due to enterococci are usually treated with combinations of beta-lactams and aminoglycosides. The glycopeptide antibiotics vancomycin and teicoplanin constitute an alternative in case of bacterial resistance to beta-lactams or aminoglycosides or when beta-lactams are poorly tolerated by patients. The glycopeptides appeared warranted by the virtual lack of enterococcal resistance to these antibiotics (22). However, inducible resistance of enterococci to glycopeptides, often plasmid mediated, has recently been reported (9, 17, 19, 24). During the last 3 years, an accumulation of reports on resistant strains (8–10, 13, 15, 17, 24), some of which are responsible for clinical infections (20), has indicated that glycopeptide resistance has spread in enterococci. Most strains belong to the species *Enterococcus faecium* and are highly resistant to vancomycin and teicoplanin (9, 10, 17, 19). The emergence of glycopeptide resistance in this species, which is frequently resistant to beta-lactams (12), is a cause of concern and leaves few therapeutic alternatives. The need for active treatments led us to evaluate the effects of various combinations of antibiotics, including beta-lactams, gentamicin, glycopeptides, and the new lipopeptide daptomycin, against enterococci that are resistant to high levels of glycopeptides.

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MATERIALS AND METHODS

Bacterial strains. Twenty-four strains of enterococci were studied. Eleven wild strains of *E. faecium* that are highly resistant to glycopeptides (including strains BM4147, BM4152, BM4165, and BM4178 [9, 10]) were obtained as unique isolates from clinical specimens between 1986 and 1988 in three hospitals in France. The strains were isolated from the feces of patients with acute leukemia or of children with chronic intestinal pseudoobstruction, after oral decontamination (eight strains), from blood cultures (two strains), and from a wound (one strain). They were identified as enterococci by Gram staining, absence of catalase, inability to produce gas, presence of Lancefield antigen group D, and growth on 40% bile, in 6.5% sodium chloride, 0.1% methylene blue, and at pH 9.6. Species identification (5, 16) was based on the absence of reduction of potassium tellurite and tests for acid production from 50 carbohydrates in API 50 CH galleries (API, La Balme-les-Grottes, France). Eight transconjugants resistant to glycopeptides, obtained after transfer to either *E. faecium* BM4107 (three strains) or *E. faecalis* JH2-2 (five strains) and previously described (6), were included. Glycopeptide-susceptible *E. faecium* BM4107 (10), *E. faecalis* JH2-2 (7), and *E. faecium* BM4147-1, BM4152-1, and BM4178-1, which are cured derivatives of strains BM4147, BM4152, and BM4178, respectively (9, 10), were studied.

Media and antibiotics. Mueller-Hinton broth supplemented with 50 μg of calcium chloride per ml and 25 μg of magnesium chloride per ml and Mueller-Hinton agar (Diagnostics Pasteur, Marnes-la-Coquette, France) were used. All incubations were at 37°C. Drugs were supplied by their manufacturers: amoxicillin, Beecham; daptomycin and vancomycin, Eli Lilly & Co.; gentamicin, Schering Corp.; imipenem,

TABLE 1. MICs of various antibiotics against enterococcal strains

Strain (no. of isolates)	MIC range (μg/ml)						
	Amoxicillin	Daptomycin	Imipenem	Penicillin G	Piperacillin	Teicoplanin	Vancomycin
Wild *E. faecium* (11)	4–64	0.5–2	8–64	8–128	16–128	64–1,024	512–1,024
E. faecium transconjugants (3)	4	1	4	8	4	64–512	256–1,024
E. faecalis transconjugants (5)	2	1	2	4	2	128–512	>512
Glycopeptide-susceptible *Enterococcus* spp. (5)	4–32	1–2	2–32	4–64	4–64	0.5–1	2–4

Merck Sharp & Dohme; penicillin G, Bristol Laboratories; piperacillin, Lederle; and teicoplanin, Gruppo Lepetit.

In vitro susceptibility to antibiotics. The method of Steers et al. (18) with 10^4 CFU per spot was used to determine the MICs of antibiotics on solid medium. The isolates were tested for β-lactamase production with Cefinase disks (bioMérieux, Marcy l'Etoile, France).

Study of combined antimicrobial activity. Combinations of antibiotics were studied by three techniques. The double-disk synergy test was used to assess the effects of combinations of beta-lactams with vancomycin or teicoplanin on all strains. Disks impregnated with 13 beta-lactams (see Table 2) were placed on top of agar plates flooded with a bacterial suspension of 10^7 CFU/ml close to disks containing 30 μg of vancomycin or teicoplanin (15 to 25 mm between disk centers). Synergy was categorized as strong, weak, or absent, depending upon alteration of inhibition zones.

All strains were further studied by the microdilution checkerboard technique. Trays were prepared in plastic microdilution panels (Dynatech Laboratories, Inc., Alexandria, Va.) as follows. Vancomycin or teicoplanin, in serial twofold dilutions from 512 to 0.5 μg/ml for resistant strains and from 4 to 0.06 μg/ml for susceptible strains, were tested alone and in combination with amoxicillin, imipenem, penicillin G, or piperacillin in serial twofold dilutions from 64 to 1 μg/ml. The inoculum was adjusted to approximately 10^6 CFU/ml, and after incubation for 24 h, the MICs of each antibiotic, alone or in combination, were noted; fractional inhibitory concentration (FIC) indexes were calculated. Synergism was defined as a FIC index of ≤0.5, antagonism as a FIC index of >4, and addition as an FIC index between 0.5 and 4.

Time-kill curves were used to test the bactericidal activity of combinations of beta-lactams, glycopeptides, and gentamicin against 4 wild resistant strains, *E. faecium* BM4152, BM4165, BM4178, and BM4185, and two cured susceptible derivatives and the bactericidal activity of combinations of daptomycin and gentamicin against the 11 resistant clinical isolates. Overnight cultures were diluted in glass tubes containing fresh Mueller-Hinton broth to yield an inoculum of approximately 5×10^6 CFU/ml. Concentrations of amoxicillin, imipenem, penicillin G, and piperacillin of 8 μg/ml (below the MIC for the organisms) and 64 μg/ml (between 1× and 4× the MIC) were used. Teicoplanin and vancomycin were used at 8 μg/ml (below the MIC for the tested organisms except for susceptible strains; 8× and 16× and 2× to 4× the MIC, respectively). Daptomycin was used at inhibitory concentrations of 5 and 10 μg/ml, and gentamicin was used at a subinhibitory concentration of 4 μg/ml. For every drug, the concentrations used were within clinically achievable ranges. After 0, 3, 6, and 24 h of incubation at 37°C, aliquots were plated on Mueller-Hinton agar. A spiral plater (Spiral System Inc., Cincinnati, Ohio) was used for the study of daptomycin-gentamicin combinations. The agar plates were incubated at 37°C for 36 h before CFU were counted. In preliminary experiments, antibiotic carryover was ruled out by plating samples of bacterial suspensions containing 10^1 to 10^3 CFU/ml in the presence or absence of the two antibiotics alone or in combination (14). Synergism was defined as a ≥ 2 $\log_{10}$ decrease in CFU/ml between the combination and its most active component after 24 h of incubation. Antagonism was defined as a ≥ 2 $\log_{10}$ increase in CFU/ml between the combination and either drug alone. Intermediate results were interpreted as addition. Bactericidal activity was defined as a ≥ 3 $\log_{10}$ decrease in the inoculum after 24 h of incubation.

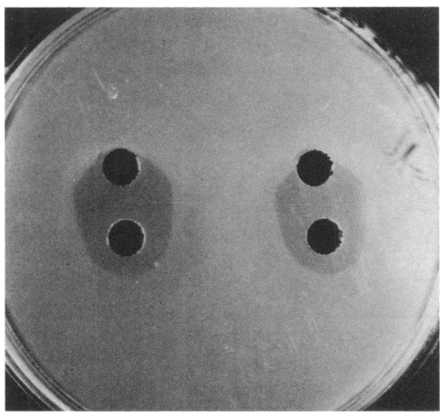

FIG. 1. Synergy between vancomycin (upper right, 30-μg disk) or teicoplanin (upper left, 30-μg disk) and penicillin G (lower left and right, 6-μg disk) against *E. faecium* BM4152.

RESULTS

Antibiotic susceptibility of the strains. The MICs of antibiotics against enterococci are summarized in Table 1. Clinical isolates of *E. faecium* and corresponding transconjugants were resistant to high levels of vancomycin (MICs ≥ 512 μg/ml) and teicoplanin (MICs ≥ 64 μg/ml). Of the 11 wild strains, 10 were resistant to penicillin G (MICs ≥ 16 μg/ml) but not by production of a β-lactamase, and none of them was resistant to high levels of gentamicin. Daptomycin was active against all the strains.

Marked synergy of combinations of beta-lactams and glycopeptides against glycopeptide-resistant enterococci. A marked synergy between beta-lactams and glycopeptides against wild strains and transconjugants was observed by the double-disk synergy test (Fig. 1 shows results of part of this analysis). Teicoplanin and vancomycin gave similar results. Beta-lactams could be divided into three groups depending upon the intensity of the synergistic effect (Table 2). Group

TABLE 2. Activity of antibiotic combinations against 19 strains of enterococci highly resistant to glycopeptides[a]

Synergy	Activity of vancomycin or teicoplanin plus indicated antibiotic (no. of strains)												
	Group 1[b]					Group 2[b]						Group 3[b]	
	Penicillin	Amoxicillin	Ticarcillin	Piperacillin	Imipenem	Oxacillin	Cephalothin	Cefoxitin	Cefuroxime	Cefotaxime	Ceftazidime	Cefsulodin	Aztreonam
Strong	16	18	17	18	5	8	6	1	2	8	6	0	0
Weak	3	1	1	0	2	6	8	5	7	6	5	0	2
Absent	0	0	1	1	2	5	5	13	10	5	8	19	17

[a] Activity tested by the double-disk diffusion technique.
[b] Group 1, Combinations with marked synergy; group 2, combinations with variable synergy; group 3, combinations with no synergy.

1 included penicillins and imipenem, which exhibited the strongest synergy when combined with glycopeptides. However, this effect was weak or absent against a strain of *E. faecium* with a penicillin G MIC of 128 μg/ml. Group 2 consisted of beta-lactams, mostly cephalosporins, which had poor activity against enterococci and displayed variable synergy. In group 3, no synergy was observed with beta-lactams considered inactive against enterococci. In conclusion, significant synergy was observed with beta-lactams most active against enterococci. By contrast, no synergy could be demonstrated between glycopeptides and beta-lactams against the glycopeptide-susceptible strains.

Synergism of combinations of amoxicillin, penicillin G, piperacillin, or imipenem with vancomycin or teicoplanin was confirmed by the microdilution checkerboard technique. The FIC indexes were below 0.5 for 10 of 11 wild *E. faecium* strains and ranged from 0.04 to 0.31 for the 8 transconjugants tested. A single strain of *E. faecium* for which the penicillin G MIC was 128 μg/ml displayed FIC indexes from 0.25 to 0.75. By contrast, for the corresponding *E. faecalis* transconjugant, for which the penicillin G MIC was 8 μg/ml, the FIC indexes were ≤0.25. Concentrations as low as 8 μg of either glycopeptide per ml and of 4 μg of every beta-lactam per ml inhibited the growth of 18 of the 19 enterococci

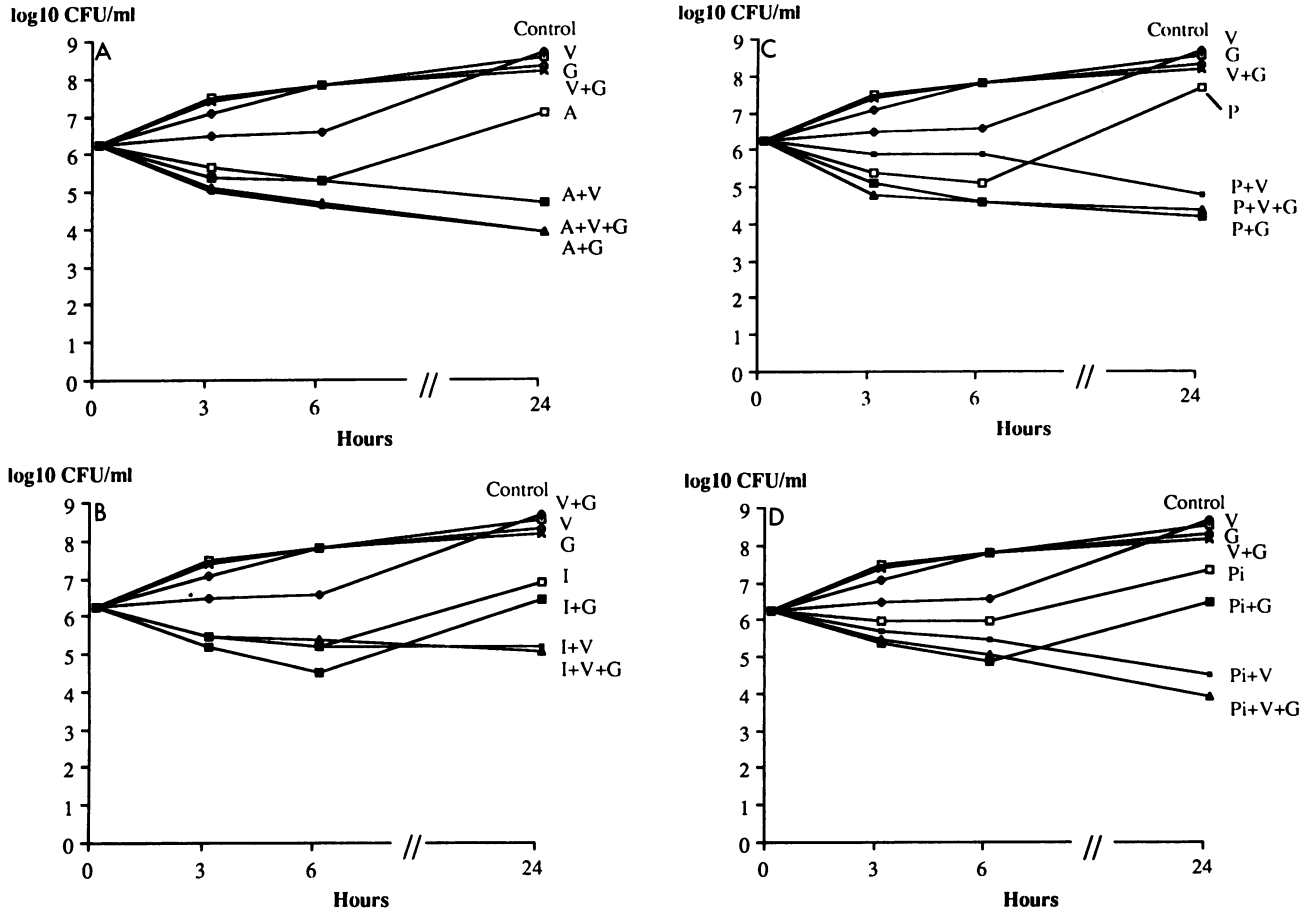

FIG. 2. Effect of combinations of low concentrations of amoxicillin, gentamicin, imipenem, penicillin G, piperacillin, and vancomycin against *E. faecium* BM4165. The control was antibiotic-free medium. Abbreviations: A, amoxicillin (8 μg/ml); G, gentamicin (4 μg/ml); I, imipenem (8 μg/ml); P, penicillin G (8 μg/ml); Pi, piperacillin (8 μg/ml); V, vancomycin (8 μg/ml). (A) Amoxicillin combinations; (B) imiperem combinations; (C) penicillin combinations; (D) piperacillin combinations.

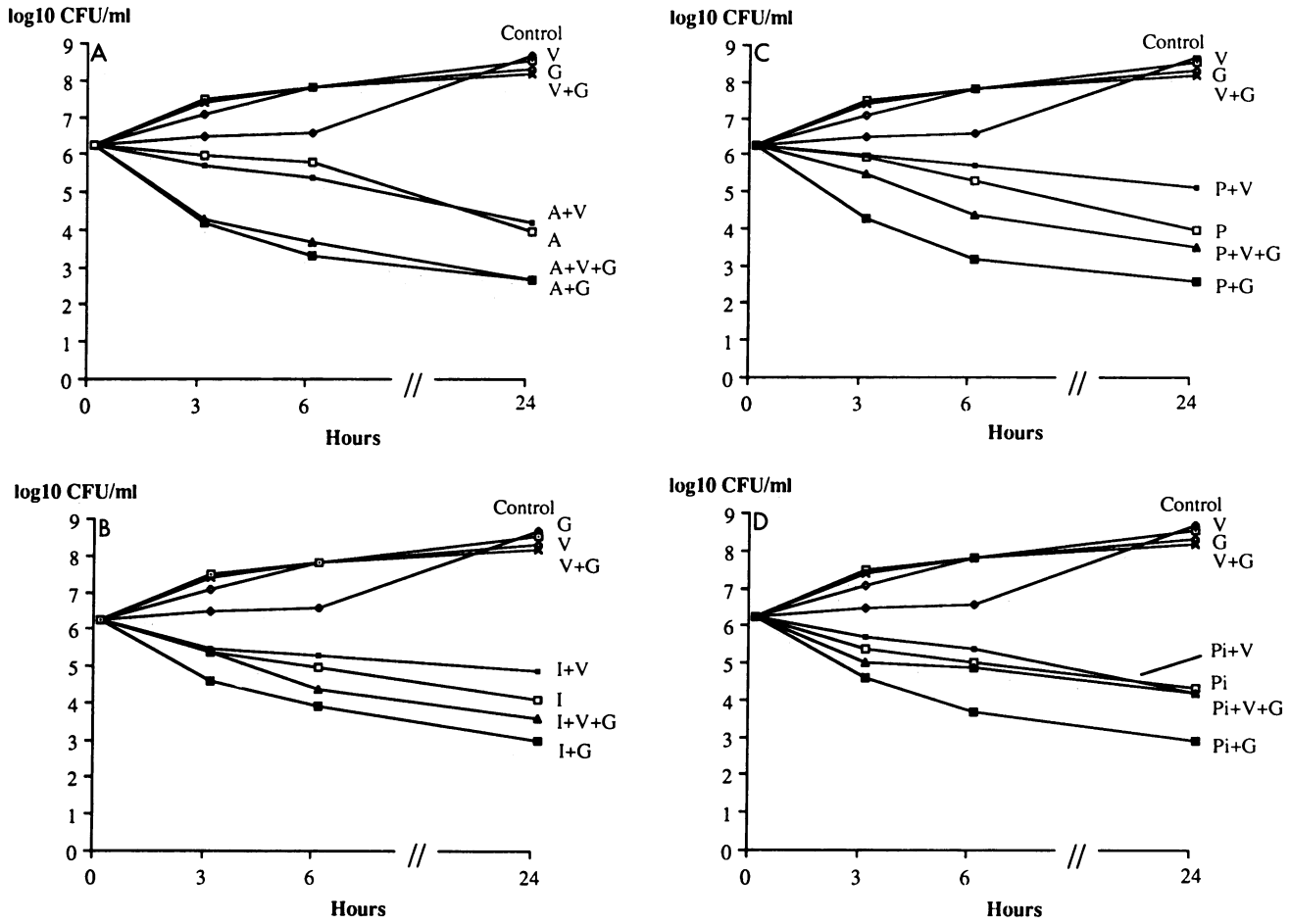

FIG. 3. Effect of combinations of high concentrations of amoxicillin, gentamicin, imipenem, penicillin G, piperacillin, and vancomycin against *E. faecium* BM4165. The control was antibiotic-free medium. Abbreviations: A, amoxicillin (64 μg/ml); G, gentamicin (4 μg/ml); I, imipenem (64 μg/ml); P, penicillin G (64 μg/ml); Pi, piperacillin (64 μg/ml); V, vancomycin (8 μg/ml). (A) Amoxicillin combinations; (B) imiperem combinations, (C) penicillin combinations; (D) piperacillin combinations.

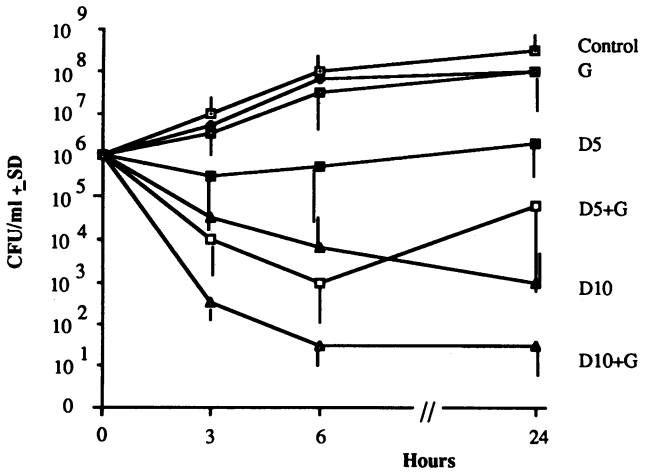

FIG. 4. Effect of combinations of daptomycin and gentamicin against 11 wild strains of *E. faecium*. Curves represent mean values of bacterial counts; standard deviations are indicated by vertical bars. The control was antibiotic-free medium. Abbreviations: D5, daptomycin (5 μg/ml); D10, daptomycin (10 μg/ml); G, gentamicin (4 μg/ml).

studied. Against the five glycopeptide-susceptible strains, combinations of beta-lactams and glycopeptides were always additive (FIC indexes, 1 to 1.5).

Time-kill curves. The bactericidal activity of combinations of amoxicillin, imipenem, penicillin G, or piperacillin with vancomycin, teicoplanin, and/or gentamicin against four wild strains with vancomycin and teicoplanin MICs of 512 to 1,024 μg/ml was tested by time-kill curves. MICs of the beta-lactams against the four strains ranged from 16 to 64 μg/ml. The results obtained for the combinations containing vancomycin against *E. faecium* BM4165 are presented in Fig. 2 and 3. The results of the combinations including vancomycin, gentamicin, and penicillin or ampicillin against the four strains are shown in Table 3. Inhibition of bacterial growth by combinations of a subinhibitory concentration of amoxicillin, imipenem, penicillin G, or piperacillin (8 μg/ml) with 8 μg of vancomycin or teicoplanin per ml was confirmed. However, the combinations were not bactericidal. Higher concentrations of every beta-lactam (64 μg/ml), alone or in combination with vancomycin or teicoplanin at 8 μg/ml were also not bactericidal. At these concentrations, the mean magnitude of killing by penicillin G, amoxicillin, and piperacillin was decreased 0.5 to 1.5 $\log_{10}$ (range, 0 to 2 $\log_{10}$) by vancomycin or teicoplanin. This effect was observed after

TABLE 3. Effect of combinations of ampicillin, gentamicin, penicillin, and vancomycin against wild *E. faecium* strains highly resistant to glycopeptides

Strain and time (h)	Magnitude of increase in killing by drugs alone or in combination (log_{10} CFU/ml)[a]																	
	Control	A8	A64	P8	P64	G	V	VA8	VA64	VP8	VP64	VG	A8G	A64G	P8G	P64G	A8VG	A64VG	P8VG	P64VG
BM4178																				
3	−1.40	−0.20	+0.50	−0.70	+1.30	−1.1	−0.40	+0.30	+0.40	+0.28	+0.30	−0.40	+1.40	+1.85	+0.60	+1.65	+1.45	+1.70	+0.76	+1.80
6	−1.54	−0.24	+0.60	−0.90	+1.35	−1.3	−1.06	+0.40	+0.52	+0.52	+0.87	−1.24	+1.60	+2.12	+1.00	+1.73	+1.90	+1.90	+1.00	+1.90
24	−1.95	−0.95	+1.60	−2.18	+2.60	−1.8	−1.48	+0.82	+1.30	+1.82	+1.70	−1.40	+2.05	+2.76	+2.10	+2.60	+2.52	+2.12	+1.90	+2.12
BM4165																				
3	−1.22	+0.63	+0.28	+0.88	+0.33	−1.12	−0.82	+0.88	+0.58	+0.38	+0.28	−0.22	+1.28	+2.08	+1.18	+1.98	+1.18	+1.98	+1.48	+1.30
6	−1.52	+1.00	+0.48	+1.18	+1.00	−1.52	−1.52	+1.00	+0.88	+0.38	+0.57	−0.32	+1.50	+2.98	+1.68	+3.08	+1.50	+2.58	+1.68	+1.88
24	−2.30	−0.93	+2.30	−1.40	+2.30	−1.92	−2.07	+1.60	+2.08	+1.48	+1.18	−2.40	+2.90	+3.58	+2.08	+3.68	+2.90	+3.58	+1.90	+2.78
BM4152																				
3	−1.06	+0.50	+1.30	+1.56	+1.17	−1.12	+0.04	+0.70	+0.50	+1.56	+0.25	+0.25	+1.00	+1.90	+1.04	+1.87	+0.70	+1.10	+0.87	+1.04
6	−1.26	−0.60	+1.85	+1.34	+1.56	−1.52	+0.04	+0.80	+0.89	+1.87	+0.45	+0.14	+1.20	+2.30	+1.25	+1.94	+1.00	+1.20	+1.04	+1.25
24	−1.85	−0.90	+2.85	−1.06	+2.84	−1.92	−0.96	+1.85	+0.80	+2.26	+0.80	−1.66	+1.80	+2.76	+2.56	+3.64	+1.90	+1.90	+1.25	+1.64
BM4185																				
3	−1.30	+0.10	+0.50	−0.08	+0.40	−1.10	−0.60	+0.50	+0.70	+0.45	+0.52	−0.30	+0.90	+1.70	+0.44	+1.30	+1.00	+1.40	+1.22	+1.30
6	−1.90	+0.30	+1.30	−0.70	+1.15	−1.70	−0.70	+1.10	+1.10	+0.92	+1.00	−0.85	+1.80	+2.50	+1.52	+2.40	+1.40	+1.70	+1.60	+2.00
24	−1.90	−1.50	+1.90	−1.64	+1.70	−1.85	−1.78	+1.40	+1.30	+1.70	+1.60	−1.60	+2.51	+3.50	+2.30	+3.60	+2.25	+3.10	+2.30	+3.22

[a] Abbreviations: A8, ampicillin, 8 μg/ml; A64, ampicillin, 64 μg/ml; G, gentamicin, 4 μg/ml; P8, penicillin G, 8 μg/ml; P64, penicillin G, 64 μg/ml; V, vancomycin, 8 μg/ml.

3, 6, and 24 h and was antagonistic for *E. faecium* BM4152 with ampicillin, penicillin G (Table 3), and piperacillin. The effect of combinations of beta-lactams with gentamicin was compared with that of beta-lactam–glycopeptide combinations. Combinations of 8 μg of penicillin G or amoxicillin per ml with 4 μg of gentamicin per ml inhibited bacterial growth but were not bactericidal (Fig. 2A and C; Table 3), whereas 8 μg of piperacillin or imipenem per ml combined with gentamicin (4 μg/ml) could not prevent bacterial growth (Fig. 2B and D; data not shown). Combinations of 64 μg of penicillin G or ampicillin per ml with 4 μg of gentamicin per ml were not bactericidal against one and two strains, respectively (Table 3), despite the absence of high-level resistance to gentamicin. For the other strains, the bacterial counts were reduced by 3.5 to 3.7 log_{10} after 24 h. Addition of gentamicin prevented the bacterial regrowth observed for one strain with imipenem after 24 h (data not shown); the combination was bactericidal against two other strains. Similarly, gentamicin-piperacillin combinations were bactericidal against two strains. Addition of gentamicin (4 μg/ml) to a combination of vancomycin (8 μg/ml) and 64 μg of amoxicillin, penicillin G, or piperacillin per ml slightly enhanced the bactericidal activity (Fig. 3A, C, and D; Table 3), but the triple combinations were sometimes slightly less effective than beta-lactam–gentamicin, with a difference of 0 to 2 log_{10} CFU/ml at 24 h. Similar results were obtained with teicoplanin. Addition of 4 μg of gentamicin per ml to vancomycin or teicoplanin did not inhibit bacterial growth (Fig. 2A). Antagonism between penicillin or ampicillin (64 μg/ml) and glycopeptides was also observed for the two bacterial derivatives susceptible to glycopeptides (data not shown). Combinations of glycopeptides with piperacillin (64 μg/ml) tended to be antagonistic, whereas those with imipenem (64 μg/ml) were additive for the two strains.

Effects of daptomycin-gentamicin combinations. The results of combinations of daptomycin with gentamicin (4 μg/ml) are shown in Fig. 4. Daptomycin at 5 μg/ml was not bactericidal against the 11 strains studied. However, a 2.8 log_{10} reduction in the inoculum was observed with 10 μg of daptomycin per ml. Killing of enterococci by daptomycin (10 μg/ml) plus gentamicin occurred after 6 h.

DISCUSSION

Antimicrobial combinations are of particular interest in treating infections caused by organisms resistant to clinically achievable concentrations of single drugs. This approach, based on the synergism displayed by certain antibiotic associations, has been successfully used for the treatment of enterococcal endocarditis by penicillins and aminoglycosides. However, *E. faecium* is often less susceptible to penicillins than *E. faecalis* and is not always killed by combinations of beta-lactams and aminoglycosides (11). In most cases, only combinations including gentamicin (or streptomycin) display synergism against this species (11). Alternative therapy is limited to glycopeptides, often combined with aminoglycosides. Resistance of enterococci to high levels of glycopeptides precludes the use of combinations of these antibiotics with aminoglycoside because of lack of inhibitory activity (Fig. 2A). Against strains of *E. faecium* resistant to low levels of vancomycin, synergy of the glycopeptide with aminoglycosides is also abolished (15). However, and most surprisingly, there is marked synergism between penicillins or imipenem and glycopeptides against the enterococci highly resistant to glycopeptides (Fig. 1; Tables 2 and 3). Despite their low susceptibility to penicillins

and high-level resistance to glycopeptides, growth of 18 of the 19 strains studied was inhibited at clinically achievable concentrations of the drugs. The calculated FIC indexes were particularly low. No significant difference was observed between amoxicillin, imipenem, penicillin G, or piperacillin combined with teicoplanin or vancomycin.

The mechanism of synergistic activity of beta-lactams and glycopeptides against enterococci resistant to the latter antibiotics is still unclear. In enterococci that are highly resistant to glycopeptides, induction of resistance correlates with synthesis of a membrane-associated protein of ca. 40 kDa (13, 17). A mutant which was more resistant to glycopeptides appeared more susceptible to penicillin G and amoxicillin than did the inducible parental strain (3). A similar observation with a mutant overproducing the resistance protein derived from an *E. faecium* strain resistant to low levels of vancomycin has already been reported (2, 24). We observed synergism between beta-lactams and glycopeptides only against glycopeptide-resistant enterococci. The presence and absence of synergism correlated with acquisition and loss, respectively, of glycopeptide resistance by the strains. These findings indicate that synergism with beta-lactams occurs only when glycopeptide resistance is expressed, after either induction or hyperproduction or the resistance protein. Al-Obeid et al. (2) have proposed that enterococcal resistance to glycopeptides is due to binding of the inducible protein to peptidoglycan precursors followed by enzymatic modification of the target of the antibiotics. According to this hypothesis, modification of the pentapeptide impairs synthesis of peptidoglycan by the usual penicillin-binding proteins. Inhibition by penicillins of other penicillin-binding proteins involved in peptidoglycan synthesis would then explain the synergism observed. Glycopeptides could therefore lower the MICs of penicillins or imipenem. Alternatively, the glycopeptide resistance protein could be directly inhibited by penicillins because of high affinity for these drugs. Binding of penicillins would, in this case, decrease the MICs of glycopeptides. However, this hypothesis seems unlikely since the penicillin-binding protein profiles of glycopeptide-resistant and -susceptible *E. faecium* strains are similar (9, 24).

The favorable effect of penicillin-glycopeptide combinations was detected by techniques that explore the bacteriostatic activity of antibiotics (Fig. 1; Tables 2 and 3). However, the bactericidal activity of higher concentrations of penicillins was antagonized in most cases by glycopeptides (Fig. 3). This effect was observed with both glycopeptide-resistant and -susceptible enterococci and is therefore more likely to be due to the action of glycopeptides than to the presence of the resistance protein. Antagonistic interactions between amoxicillin and vancomycin or teicoplanin have been reported (4). It remains to be determined whether the therapeutic benefit of the marked MIC decrease of the drugs in combination is abolished by the suppression by glycopeptides of the bactericidal effect of penicillins. Addition of gentamicin, in the absence of high-level resistance to this drug, improved the bactericidal activity of combinations of glycopeptides and beta-lactams. This triple association deserves further evaluation in animal models.

Daptomycin is active against glycopeptide-resistant enterococci (9, 10), and a combination of this antibiotic (10 μg/ml) with gentamicin appeared synergistic and bactericidal after 6 h (Fig. 4). Similar results were previously obtained with glycopeptide-susceptible enterococci (21, 23). This combination therefore appears to be of interest since it is active against both glycopeptide-resistant and -susceptible enterococci. Lack of cross-resistance between glycopeptides and lipopeptides probably results from a difference in the mode of the two groups of antibiotics (1).

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